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(71) Applicant: NEW ENGLAND DEACONESS HOSPITAL CORPORATION [US/US]; 185 Pilgrim Road, Boston, MA 02215 (US).

(72) Inventors: LEES, Robert, S. ; LEES, Ann, M. ; 203 Clinton Road, Brookline, MA 02146 (US). SHIH, Ing, Lung ; 39 Chaske Avenue, Auburndale, MA 02166 (US). FISCHMAN, Allan ; 1 Longfellow Place, Boston, MA 02114 (US).

(74) Agents: LAPPIN, Mark, G. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

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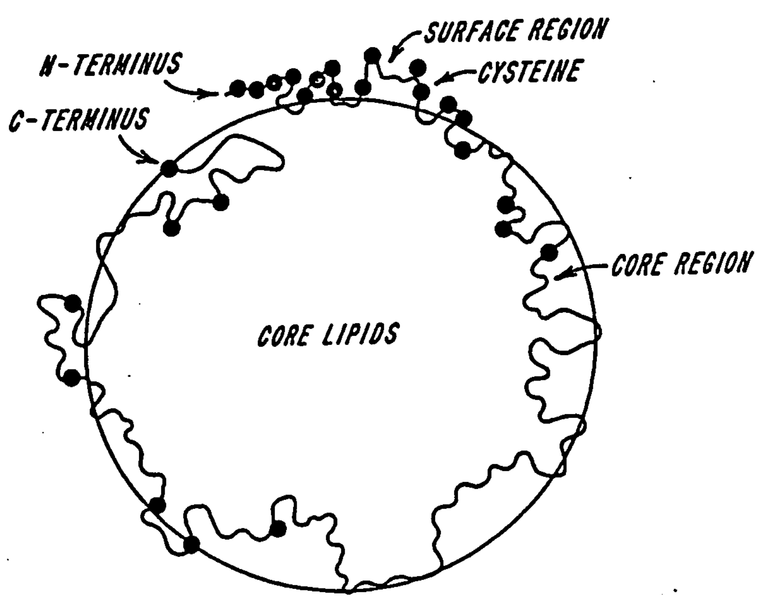

(57) Abstract

Vascular disease including asymptomatic atherosclerosis can be diagnosed by administering a synthetic peptide to a patient and then detecting the location of the peptide which has accumulated within the patient's vascular system. The synthetic peptides have an affinity for, and propensity to accumulate at a site of arterial injury. Specific examples of such peptides have molecular conformations or amino acid sequences that are homologous to portions of LDL. The synthetic peptide can be labelled with, for example, a radioisotope or paramagnetic contrast agent, thereby enabling the extracorporeal detection of vascular disease within the patient.

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SYNTHETIC PEPTIDES FOR ARTERIAL IMAGING

BACKGROUND OF THE INVENTION

The U.S. Government has rights in this invention pursuant to NIH Grant No. HL32975.

The technical field of this invention concerns methods and means useful for the early detection of vascular disease, such as atherosclerosis, and in particular, methods and means employing labelled synthetic peptides to detect arterial injury.

Atherosclerosis is a disease which causes the thickening and hardening of the arteries, particularly the larger artery walls. It is characterized by lesions or raised fibrous plaques which form within the arterial lumen. The plaques are most prevalent in the abdominal aorta, coronary arteries or carotid arteries and they increase progressively with age. They commonly present dome-shaped, opaque, glistening surfaces which bulge into the lumen. A lesion typically will consist of a central core of lipid and necrotic cell debris, capped by a collagen fibromuscular layer. Complicated lesions will also include calcified deposits and exhibit various degrees of necrosis, thrombosis and ulceration.

The injury at, or deformities of, the arterial lumen presented by the plaque and associated deposits result in occluded blood flow, and ultimately in angina, cerebral ischemia, renal hypertension, ischemic heart disease, stroke, and diseases of other organs, if untreated. At present, coronary atherosclerosis is still the leading cause of death in the United States, claiming the lives of over a half million Americans annually, roughly twice as many as are killed by cancer.

Unfortunately, there are no existing diagnostic methods which can detect the early stages of atherosclerosis and related vascular diseases which often are clinically silent. Since lifestyle changes, drug therapy and other means exist for delaying or reducing vascular occlusion or the stresses on various body organs which result from atherosclerotic lesions, the early detection of atheromatous plaques in the vascular system would be of considerable value in permitting preventive intervention at a time when it can be most effective.

Arteriography, the conventional approach to diagnosing vascular disease, involves catheterization and the injection of radioopaque substances into the bloodstream in order to image obstructions in the arteries. This procedure involves significant morbidity, in that infection, perforation of the artery, arrhythmia, stroke, infarction and even death can occur. Because of the risks involved, arteriograms typically are reserved for individuals with advanced or acute atherosclerotic disease.

A variety of less invasive techniques for the diagnosis of vascular injury and disease have been proposed. These techniques include plethysmography, thermography and ultrasonic scanning (Lees and Myers, Adv. Int. Med. (1982) 27:475-509).

Other non-invasive approaches to the diagnosis of vascular injury which have been proposed by the present inventor involve the administration of labelled target-seeking biologically active molecules or antibodies which preferentially seek out arterial lesions (U.S. Patent Application Ser. No. 929,012, entitled "Detection of Vascular Disease", filed Nov. 10, 1986), and the administration of labelled low density lipoproteins (LDLs) to the vascular system of a patient (U.S. Patent Nos. 4,647,445 and 4,660,563). LDLs circulating in the blood are known to bind to atherosclerotic plaques (Lees et al. (1983) J. Nucl. Med. 24:154-156). In mammals this binding most likely occurs via apolipoprotein B-100 (apo B-100), the protein moiety of the LDL molecule, which is responsible for the removal of LDL from the circulation by receptor-mediated uptake in a variety of cell types. LDLs conjugated to a radioactive label can be used to provide information on the location and extent of plaque in the vascular system by imaging them with a radiation detector. Alternatively, LDLs can be labelled with a non-radioactive, paramagnetic contrast agent capable of detection in magnetic resonance imaging (MRI) systems.

One disadvantage to this method is that several days are typically required to isolate LDLs from the patient's blood and to label them. Often, such a delay in diagnosis and subsequent treatment is detrimental for critically ill patients. Further, an additional risk of viral infection is incurred if donor blood is employed as an LDL source.

Consequently, there exists a need for better non-invasive techniques and reagents capable of detecting and mapping early, non-stenosing, non-flow-disturbing atherosclerotic arterial lesions.

Accordingly, it is an object of the present invention to provide synthetic peptides which are useful for imaging vascular disease.

It is another object of the invention to provide a synthetic peptide useful for imaging which is inexpensive and easy to prepare.

It is yet another object of the invention to provide an improved method of detecting and mapping vascular injury, including vascular injury at its early stages.

Yet another object of the present invention is to provide a method, which is non-invasive, of detecting and mapping vascular injury.

SUMMARY OF THE INVENTION

It has now been discovered that vascular diseases, including asymptomatic atherosclerosis, can be diagnosed by administering a labelled synthetic peptide to a patient, and then detecting the location, pattern, and concentration of the peptide which has accumulated within the patient's vascular system.

Accordingly, the invention is directed to molecules having an affinity for a site of arterial injury. More specifically, the invention is directed to synthetic peptides which are useful for detecting and imaging this injury.

The synthetic peptides administered have an affinity for, and propensity to accumulate at a site of arterial injury, for example, sustantially the same affinity and propensity as does LDL. In addition, the synthetic peptides may also have a molecular conformation (or size, shape, and charge) which is substantially analogous to a portion of the molecular conformation of LDL, for example. Moreover, the synthetic peptide may have an amino acid sequence sufficiently duplicative of that of at least a portion of the apo B moiety of LDL, such that the peptide accumulates at a site of arterial injury in a manner characteristic of LDL. Examples of particularly useful sequences are:

(NH_2)-Y-R-D-K-E-S-D-G-E-T-I-K-I-(COOH)

and

(NH_2)-Y-R-A-L-V-D-T-L-K-F-V-T-Q-A-E-G-A-K-(COOH).

The synthetic peptide may be linked to a label to enable its monitoring within the patient. Preferable labels include a radioisotope such as ^{131}I, ^{125}I, ^{123}I, ^{111}In, ^{99m}Tc, ^{203}Pb, ^{198}Hg, or ^{201}Tl, or a paramagnetic contrast agent. Such labels may enable the extracorporeal monitoring of synthetic peptide within the vascular system of the subject with, for example, a gamma scintillation camera or an MRI system.

The synthetic peptides are useful for detecting and imaging arterial injury in the vascular system of a subject.

A preferred detection method includes introducing into a subject a synthetic peptide of the form set forth above. The peptide to be introduced may be administrated by arterial or venous injection. Alternatively, a non-hydrolyzable derivative may be administered orally or nasally. The introduced synthetic peptide is then allowed to circulate within the vascular system of the subject, whereby at least a portion of it accumulates at a site of arterial injury. The portion of the synthetic peptide which has accumulated at a site of arterial injury is then detected. The detection step may further include imaging the region of the

subject's vascular system at which the synthetic peptide has accumulated.

An additional step of quantitating the amount of synthetic peptide which has accumulated at a site of vascular injury may also be carried out as part of the method of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description when read together with the accompanying drawings in which:

FIG. 1 shows a schematic model of apo B-100 configuration on the LDL molecule and surface-exposed regions;

FIG. 2 shows a representative plasma decay curve for ^{125}I-labelled synthetic peptide; and

FIG. 3 is an onlay autoradiogram of the abdominal aorta of a rabbit treated with ^{125}I-labelled synthetic peptide SP-4, showing labelled healing lesions in the balloon de-endothelialized section of the aorta (B) as compared with the unlabelled control portion (A).

DESCRIPTION OF THE INVENTION

This invention provides synthetic peptides which have affinity for, and the propensity to accumulate at a site of arterial injury, and therefore are useful in detecting, diagnosing and monitoring vascular disease.

Specific examples of such synthetic peptides having these characteristics may have an amino acid sequence that is analogous to portions of known polypeptides which have an affinity for a site of vascular injury, i.e., have a molecular conformation, charge, and/or size which is similar to that part of the polypeptide (e.g., LDL) which is responsible for its affinity for arterial lesions. Alternatively, the synthetic peptides of the present invention may be homologous with portions of the apo B-100 protein moiety of LDL. The primary structure of apo B-100 has become available by virtue of its cloning (Knott et al. (1986) Nature 323:734-742; Lusis et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:4597-4601; Carlsson et al. (1985) Nucl. Acids Res. 13:8813-8826) Further, enzymatic treatment of apo B-100 with trypsin has enabled the identification of surface regions of LDL, and which therefore appear to be involved in the binding of LDL to various cells and tissues (Forgez et al. (1986) Biochem. Biophys. Res. Comm. 140:250-257; Knott et al. (1986) Nature 323:734-742). The amino acid sequence analyses of such tryptic peptides are shown in TABLE 1.

TABLE 1*

HPLC Fraction No.	AA Sequence	Corresponding to Apo B AA Residue Nos.
24	K-F-V-T-Q-A-E-G-A-K	1008-1016
123	K-L-P-Q-Q-A-N-D-Y-L-N-S-F-N-N-E-R	2091-2106
70	L-P-Q-Q-A-N-D-Y	2091-2098
49	K-F-R-E-T-L-E-N-T-R	2485-2493
99	R-I-S-L-P-D-F-R	2679-2685
161	R-T-F-Q-I-P-G-Y-T-V-P-V-V-N-V-E-V	3218-3236
81	Y-T-V-P-V-V-N-V-E	3237-3242
134	S-P-F-T-I-E-M-S-A-F-G-Y-V-F-P-K	3224-3232
184	R-V-P-S-Y-T-L-I-L-P-S-L-E-L-P-V-L-H-V-P-R	3265-3275
59	K-I-A-D-F-E-L-P-T-I-I-V-P-E-Q-T-I-E-I-P-S-?-I	3828-3841
106	R-N-L-Q-N-N-A-E-W-V-Y-Q-G-A-I-R	4080-4094

*(from Forgez et al., *ibid*.)

Based on this data and that of Lusis et al (*ibid*.), synthetic peptides having an amino acid sequence analogous to portions of apo B portion of LDL have been designed. Two representative peptides are (1) SP-1 and (2) SP-4 shown below:

(1) (NH_2)-Y-R-D-K-E-S-D-G-E-T-I-K-I-(COOH).

(2) (NH_2)-Y-R-A-L-V-D-T-L-K-F-V-T-Q-A-E-G-A-K-(COOH)

These peptides have substantially the same affinity for, and propensity to accumulate at a site of arterial injury as LDL. Such peptides can be synthesized by any number of established procedures, including, for example, the expression of a recombinant DNA encoding that peptide in an appropriate host cell.

These peptides can also be produced by the established procedure of solid phase peptide synthesis. Briefly, this procedure entails the sequentially assembly of the appropriate amino acids into a peptide of a desired sequence while the end of the growing peptide is linked to an insoluble support. Usually, the carboxyl terminus of the peptide is linked to the polymer from which it can be liberated upon treatment with a cleavage reagent.

The peptides so synthesized are then labelled with a reagent which enables the monitoring of the peptide after its administration to a patient. In the preferred embodiments of the invention, synthetic peptides having an amino acid sequence homologous to, or having at least a part of their molecular conformation being substantially analogous to, surface portions of native apo B-100 are used to create radiolabelled diagnostic reagents. The label may be, for example, a radioisotope such as ^{125}I or ^{99m}Tc, both of which may be imaged extracorporeally by radiation detection means such a gamma scintillation camera.

Alternatively, the synthetic peptides can be labelled with a non-radioactive, paramagnetic contrast agent capable of detection in MRI systems. In such systems, a strong magnetic field is used to align the nuclear spin vectors of the atoms in a patient's body. The field is then disturbed and an image of the patient is read as the nuclei return to their equilibrium alignments. In the present invention, synthetic peptides can be linked to paramagnetic contrast agents such as gadolinium, cobalt, nickel, manganese or iron complexes, to form conjugate diagnostic reagents that are imaged extracorporeally with an MRI system.

The labelled synthetic peptide is then administered to a patient. Administration may be accomplished by arterial or venous injection. Alternatively a non-hydrolyzable derivative of the peptide (e.g., a keto methylene derivative) may be administered by mouth. Further, administration may be accomplished nasally.

Preferably, the amount of labelled peptide administered is sufficient for later detection. The rabbit model has been imaged both by onlay autoradiography with ^{125}I-labelled LDL and by external imaging with ^{99m}Tc-labelled LDL using a gamma scintillation camera. Human lesions have been imaged with both isotopes using a gamma scintillation camera. In each case, onlay autoradiography of the excised rabbit aorta has been reliably predictive of the _in vivo_ results with extracorporeal imaging.

For example, it is known that LDL accumulates both in the balloon de-endothelialized healing arterial wall of the rabbit and in human atheroma (Camejo (1982) Adv. Lipid Res. 19:1-53). Accordingly, a rabbit whose abdominal aorta has been balloon de-endothelialized approximately four weeks prior is used as a test model for human arterial disease. However, other animals or experimental systems can be used as well.

In preparation for vascular administration, each labelled synthetic peptide may be bound to the surface of a lipid emulsion such as a cholesterol ester phospholipid microemulsion. The emulsion is then injected intravenously into the rabbit. Approximately twenty-four hours later, the rabbit is subjected to extracorporeal monitoring appropriate for the specific label on the peptide. Alternatively, the rabbit is sacrificed, and its aorta removed and washed. The aorta is then either cut into sequential portions which are then monitored in a liquid scintillation counter, or is dried, covered with a layer of polyester wrap, and placed on a sheet of x-ray film which is then developed to produce an onlay autoradiogram after suitable storage time in the dark.

An example of such an autoradiogram is shown in FIG. 3. in which the ^{125}I-labelled synthetic peptide, SP-4 was used to image arterial injury.

The invention will next be described in connection with certain preferred embodiments;

however, it should be clear that various changes, additions and subtractions can be made without departing from the spirit or scope of the invention.

1. Peptide Synthesis:

Synthetic peptides are designed from the amino acid sequences of various portions of apo B which are shown in FIG. 1. Two representative peptides are (1) SP-1 and (2) SP-4 shown below:

(1) (NH_2)-Y-R-D-K-E-S-D-G-E-T-I-K-I-(COOH).

(2) (NH_2)-Y-R-A-L-V-D-T-L-K-F-V-T-Q-A-E-G-A-K-(COOH)

Peptides are synthesized by solid phase peptide synthesis according to the established method of Stewart and Young (<u>Solid Phase Peptide Synthesis</u>, 2nd ed., (1984) The Pierce Chemical Co., Rockford, IL pp. 53-123), herein incorporated as reference. In the preferred embodiment, the schedule listed below in TABLE 2 is followed, but any one of the other schedules listed in this reference may alternatively be used to generate the desired peptides.

TABLE 2

SCHEDULE FOR SOLID PHASE PEPTIDE SYNTHESIS
(Dioxane-HCl Deprotection: DCC* Coupling)

Step	Reagent	No. Repeats	Vol(ml)	Time(min)
1	dry CH_2Cl_2	4	25	1
2a	50% TFA**	1	25	1
2b	50% TFA	1	25	20
3	dry CH_2Cl_2	2	25	1
4	dry 2-propanol	2	25	1
5	CH_2Cl_2	3	25	1
6	5% DIEA°	1	25	2
7	CH_2Cl_2	2	25	1
8	5% DIEA	1	25	2
9	CH_2Cl_2	5	25	1
10	Introduce symmetric anhydride of Boc AA°°	1	20	20
11	TFE#/DIEA/ CH_2Cl_2(add)	1	5	10
12	CH_2Cl_2	3	25	1
13	100% EtOH	3	25	1

*dicyclohexylcarbodiimide
**trichloroacetic acid
°diisopropylethylamine
°°tert-butyloxycarbonyl amino acid
#2,2,2-trifluoroethanol

2. Radiolabelling:

A. Labelling with ^{125}-Iodine

Iodination of LDL (for comparative use) and synthetic peptides SP-1 and SP-4 is performed by a previously described modification of the McFarlane iodine monochloride technique (Lees and Lees (1983) Proc. Natl. Acad. Sci. USA 80:5098-5103). The radiolabelled lipoprotein or synthetic peptide is separated from unbound radioisotope by passage through a gel filtration "desalting" column of Sephadex G-25 or the equivalent.

B. Labelling with ^{99m}Tc

The synthetic peptides may be labelled directly with technetium (Tc), or indirectly through covalent attachment of a chelating group such as diethylenetriamine pentaacetic acid (DTPA), which is known to chelate a variety of metals including radioisotopes such as ^{111}-indium.

i. Direct Coupling to ^{99m}Tc

50 mCi ^{99m}Tc (in the form of $^{99m}TcO_4$-), in a 0.5 ml aqueous solution, is added to 1-6 mg but preferrably to 2 mg SP-1 or SP-4, in 0.5 ml of a 0.2 M sodium bicarbonate, pH 8.0, and mixed thoroughly for 10 minutes at room temperature. The pH is raised to 8.0 - 9.0 if necessary with 0.25 M sodium hydroxide. To the mixture is then added 10 mg of reduced sodium dithionite (57.5 mmoles) freshly

dissolved in 0.5 ml distilled water. The mixture is gently stirred for 30 minutes at room temperature.

The radiolabelled synthetic peptide fraction radiolabelled with ^{125}I or ^{99m}Tc is separated from uncoupled technetium and sodium dithionite by molecular sieve chromatography. A 1 x 50 cm column of Sephadex G-25, equilibrated with a bicarbonate-EDTA buffer (0.2 M sodium bicarbonate, pH 8.0, 0.001 M EDTA), is suitable for separation. The column is standardized with blue dextran and potassium iodide to determine the void volume and the column volume, respectively. The reaction mixture is applied to the column, and bicarbonate-EDTA buffer is used to elute column fractions. The macromolecular radioactive peak that elutes at a position characteristic for the synthetic peptide is isolated and is ready for use.

ii. Indirect Coupling to ^{99m}Tc

A chelating ligand (e.g., DTPA as per Hnatowich et al. (1983) Science 220:613, or bromoacetylparaaminobenzyl EDTA (BABE) as per Meares et al. (1984) Analyt. Biochem. 142:142-686) is covalently bound to the N-terminus of the peptide. Technetium is then chelated to the DTPA- or BABE-synthetic peptide by the procedure described above for direct labelling of synthetic peptide. Technetium, in the form of $^{99m}TcO_4^-$, is added to the DTPA-synthetic peptide, and to the mixture is added a solution of reduced sodium dithionite, pH 8.0-9.0. ^{99m}Tc-labelled DTPA-synthetic peptide

is separated from uncomplexed ^{99m}Tc and sodium dithionite by column chromatography.

These preparations are then characterized by silica gel chromatography essentially as described by Meares et al. (ibid.) and by HPLC.

The ^{99m}Tc-labelled peptide is applied neat in solution or bound to a lipid emulsion.

3. Animal Model:

Male New Zealand white rabbits (2 to 3 kg each) are obtained from ARI Breeding Labs, West Bridgewater, MA. Their abdominal aortas are denuded of endothelium by a modification of the Baumgartner technique (Roberts (1983) J. Lipid Res. 24:1160-1162). After each animal is anesthetized with ketamine and ether, the left femoral artery is isolated; a 4F Fogarty embolectomy catheter (Model 12-040-4F, Edwards Laboratories Incorporated, Santa Ana, CA) is introduced through an arterotomy in the femoral artery and is advanced under fluoroscopic visualization to the level of the diaphragm. The catheter is inflated to a pressure of about 3 psi above the balloon inflation pressure with radiographic contrast medium (Conray, Mallinkrodt, St. Louis, MO). Three passes are made through the abdominal aorta with the inflated catheter to remove the aortic endothelium before removal of the catheter, ligation of the femoral artery, and closure of the wound. The animals are allowed to heal for a period of 4 to 5 weeks before injection of the labelled synthetic peptides.

4. Injection of Labelled Synthetic Peptides:

Each labelled synthetic peptide preparation (containing, for example, 150 to 400 mCi of ^{125}I-labelled SP-1 or SP-4 bound to lipoprotein) is injected into the marginal ear vein of the ballooned and healing rabbits. Serial blood samples are obtained from the opposite ear during the ensuing 24 hours and are analyzed for radioactivity. The labelled protein concentration in the blood sample that is withdrawn 5 minutes after injection is considered as time zero radioactivity in the calculation of average plasma radioactivity. FIG. 2 shows representative plasma decay curves for ^{125}I-labelled synthetic peptides.

5. Aortic and Adrenal Specimens:

Twenty-four hours after injection of the labelled synthetic peptide preparations, each animal is injected intravenously with 4 ml of a 0.5% solution of Evans blue dye (Allied Chemical Company, National Aniline Division, NY, NY) which stains areas of de-endothelialized aorta blue. After 1 hour, the animal is sacrificed by a lethal injection of pentobarbital. After sacrifice, the aorta is removed completely, and the remaining aorta is washed in formalin.

6. Detection by Autoradiography:

The washed aortas from the animals that had been injected with labelled synthetic peptide were opened along the ventral surface. These segments are

then pinned out, immersed in 10% trichloroacetic acid, and photographed. The fixed, opened vessels are then covered with a single layer of plastic (Saran) wrap, placed on high speed x-ray film (Kodak Orthofilm OH-1), and stored for 2 to 3 weeks in a Kodak "X-Omatic cassette" (24 X 30 cm) before development in a 90 second X-OMAT.

To compare the relative accumulation of the ^{125}I-labelled synthetic peptides in the aorta and adrenal gland, it is necessary to correct for differences in mean plasma concentration of the labelled compounds. The mean concentration of synthetic peptide-associated ^{125}I radioactivity is calculated by numerical integration of the plasma decay curves and division by the time since injection of the isotope.

The audioradiograph shown in FIG. 3 demonstrates clear-cut localization of the synthetic peptide on the image at the healing (re-endothelization) edge of the aortic lesions produced by the previous trauma. Since this lesion is known to resemble human arteriosclerosis in many important respects, including accumulation of lipoproteins and other pathological changes, the ability of the synthetic peptides to localize at the trauma site, and to permit the imaging thereof demonstrates the utility of the present invention in imaging vascular disease.

What is claimed is:

Claims

1. A synthetic peptide having an affinity for, and propensity to accumulate at a site of arterial injury.

2. The synthetic peptide of claim 1 having substantially the same affinity for, and propensity to accumulate at said site of arterial injury as does low density lipoprotein (LDL).

3. The synthetic peptide of claim 2 wherein at least a part of the molecular conformation of said peptide is substantially analogous to a portion of the molecular conformation of LDL.

4. The synthetic peptide of claim 2 having an amino acid sequence sufficiently duplicative of that of at least a portion of the apolipoprotein B (apo B) moiety of LDL such that said peptide accumulates at said arterial injury in a manner characteristic of LDL.

5. The synthetic peptide of claim 1 further comprising a label linked thereto.

6. The synthetic peptide of claim 5 wherein said label is radioactive.

7. The synthetic peptide of claim 6 wherein said radioactive label is selected from the group consisting of ^{131}I, ^{125}I, ^{123}I, ^{111}In, ^{99m}Tc, ^{203}Pb, ^{198}Hg, and ^{201}Tl.

8. The synthetic peptide of claim 6 wherein said radioactive label is ^{99m}Tc.

9. The synthetic peptide of claim 5 wherein said label is a paramagnetic contrast agent.

10. The synthetic peptide of claim 4 comprising the following amino acid sequence:

(NH_2)-Y-R-D-K-E-S-D-G-E-T-I-K-I-(COOH).

11. The synthetic peptide of claim 4 comprising the following amino acid sequence:

(NH_2)-Y-R-A-L-V-D-T-L-K-F-V-T-Q-A-E-G-A-K-(COOH).

12. A method for the detection of arterial injury in the vascular system of a subject, said method comprising the steps of:

(a) introducing into said subject a synthetic peptide having an affinity for, and propensity to accumulate at a site of arterial injury;

(b) allowing said introduced synthetic peptide to circulate within said vascular system,

whereby at least a portion of said introduced synthetic peptide accumulates at said site of arterial injury; and

(c) detecting the location in said vascular

system of said introduced synthetic peptide which has accumulated at said site of arterial injury.

13. The method of claim 12 wherein said introducing step further comprises administering a synthetic peptide having substantially the same affinity for, and propensity to accumulate at said site of arterial injury as does LDL.

14. The method of claim 13 wherein said introducing step further includes administering a synthetic peptide having a molecular conformation analogous to the molecular conformation of a portion of LDL.

15. The method of claim 13 wherein said introducing step further includes administering a synthetic peptide having an amino acid sequence sufficiently duplicative of that of at least a portion of the apo B moiety of LDL, such that said peptide accumulates at said site of arterial injury in a manner characteristic of LDL.

16. The method of claim 15 wherein said introducing step further includes administering a synthetic peptide having the following amino acid sequence:

(NH_2)-Y-R-D-K-E-S-D-G-E-T-I-K-I-(COOH).

17. The method of claim 15 wherein said introducing step further includes administering a synthetic peptide having the following amino acid sequence:

(NH_2)-Y-R-A-L-V-D-T-L-K-F-V-T-Q-A-E-G-A-K-(COOH).

18. The method of claim 15 wherein said introducing step further includes administering a synthetic peptide having a label linked thereto.

19. The method of claim 18 wherein said introducing step further includes administering a synthetic peptide having a paramagnetic contrast agent linked thereto.

20. The method of claim 18 wherein said introducing step further includes administering a synthetic peptide having a radioactive label linked thereto.

21. The method of claim 20 wherein said introducing step further includes administering a synthetic peptide having one of the group consisting of ^{131}I, ^{125}I, ^{123}I, ^{111}In, ^{99m}Tc, ^{203}Pb, ^{198}Hg, and ^{201}Tl linked thereto.

22. The method of claim 21 wherein said introducing step further includes administering a synthetic peptide having ^{125}I linked thereto.

23. The method of claim 21 wherein said introducing step further includes administering a synthetic peptide having ^{99m}Tc linked thereto.

24. The method of claim 12 wherein said introducing step further comprises the administration of said synthetic peptide by arterial injection.

25. The method of claim 12 wherein said introducing step further comprises the administration of said synthetic peptide by venous injection.

26. The method of claim 12 wherein said introducing step further comprises the oral administration of said synthetic peptide.

27. The method of claim 12 wherein said introducing step further comprises the nasal administration of said synthetic peptide.

28. The method of claim 12 wherein said detecting step further includes imaging a region of said vascular system at which said synthetic peptide has accumulated.

29. The method of claim 18 wherein said detecting step further includes the extracorporeal monitoring of said labelled, synthetic peptide.

30. The method of claim 22 wherein said detecting step further includes the extracorporeal monitoring of said radioactive label with a gamma scintillation camera.

31. The method of claim 23 wherein said detecting step further includes the extracorporeal monitoring of said radioactive label with a gamma scintillation camera.

32. The method of claim 19 wherein said detecting step further includes the extracorporeal monitoring

of said paramagnetic contrast agent with a magnetic resonance imaging system.

33. The method of claim 18 further comprising the step of quantitating an amount of said detected synthetic peptide.

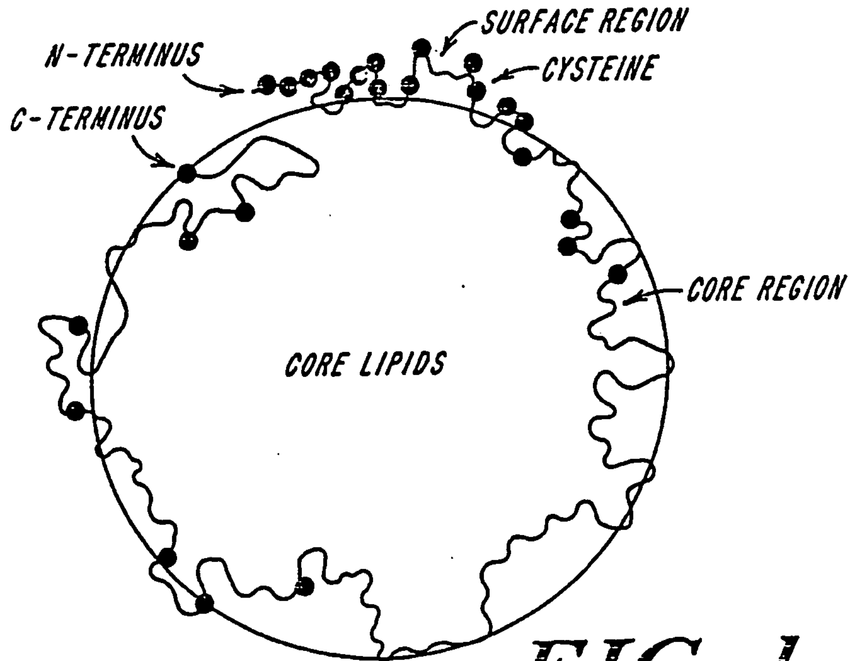
SURFACE REGION
N-TERMINUS
CYSTEINE
C-TERMINUS
CORE REGION
CORE LIPIDS

FIG. 1

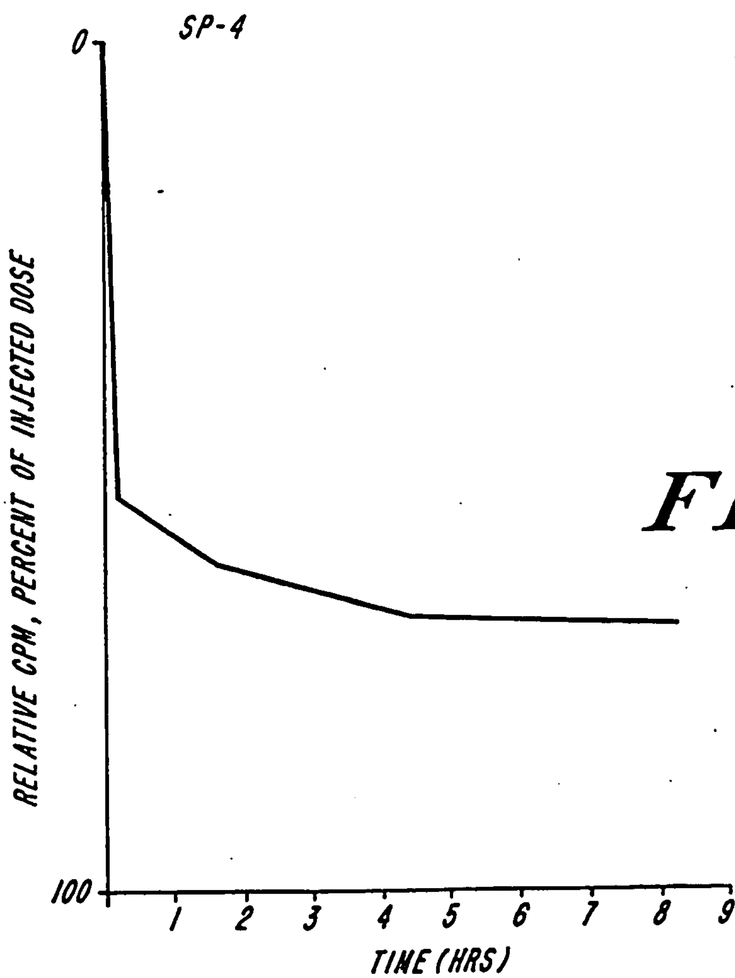
SP-4
0
100
RELATIVE CPM, PERCENT OF INJECTED DOSE
1
2
3
4
5
6
7
8
9
TIME (HRS)

FIG. 2

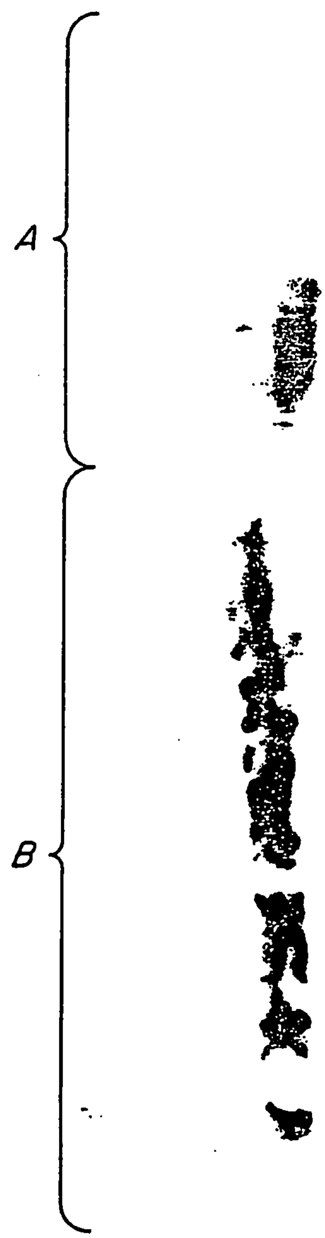

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/01854

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 49/02; C07K 7/08
US: 530/326; 530/327; 424/9, 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/326; 530/327; 424/9;424/1.1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Data bases: Chemical Abstracts Service On line (File CA, 1967-1989); Biosis; Medline: Automated Patent Search (File US PAT 1975-1989); Sequence search; See Attachment.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	N, Atherosclerosis. 1979. V. 34, pp. 391-405. Hollander, See entire article.	1-11
Y	Biochem. Biophys. Res. Comm. 15 Oct. 1986 V. 140(1), pp 250-257, Forgez, See page 254, Table I.	1-9, 11-15, 17-33
Y	N, Proc. Natl. Acad. Sci, USA, July, 1985 v. 82, pp. 4597-4610, Lusis, See Materials and Methods and Figure 2.	1-33
Y	N, Proc. Natl. Acad. Sci, USA. November 1986, V. 83 pp. 8142-8146, Law. See Figure 2.	1-9, 11-15, 17-33
Y	U.S., A, 4,668,503, Hnatowich, 26 May 1987. See abstract	5-8
Y	E.P., A, 0135125. DuPont De Nemours Co, 27 March 1985. See abstract	5,9,12 -19,24-29,32,33

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 July 1989	19 SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Nina Ossanna

International Application No. PCT/US89/01854

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	N, Journal of Nuclear Medicine, February 1983, V. 24(2) pp. 154 -156, Lees. See entire article.	12-18,20-31,33
Y	U.S.,A 4,660,563, Lees, 28 April 1987. See Abstract.	12-33
Y	Journal of Lipid Research, 1983, Vol. 24, pp1160 1167, Roberts, See entire article.	5-8,12-18, 20-28

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter[12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out[13], specifically:

3. ☐ Claim numbers__________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment to Form PCT/ISA/210, Part II.

II. Fields searched, terms

ather osclerosis (plaque, lesion) vascular (injury,

disease) diagnosis, detection

apolipoproteins or Low density lipoprotein

arterial imaging
binding
Robert S. Lees